Himachal Journal of Agricultural Research
Volume 52, Issue 1, June 2026; pp. 162–172; DOI: 10.18311/HJAR/2026/68818

INFORMATICS JOURNALS
Crop Protection

A Comprehensive Survey and Assessment of Rice Blast Disease Incidence from Major Rice Growing Districts of Himachal Pradesh, India

Akshay Pathania*, Pardeep Kumar, Twinkle and Arushi Chauhan

Department of Plant Pathology, College of Agriculture, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur – 176062, Himachal Pradesh, India; akshaypathania11.10.1995@gmail.com

Abstract

Rice blast, caused by *Pyricularia oryzae,* is a major constraint to rice production in the mid-hill ecosystems of Himachal Pradesh, India. A survey was conducted during the kharif seasons of 2022 and 2023 across major rice-growing districts of Himachal Pradesh to quantify disease incidence. Disease incidence was recorded at maximum tillering to panicle initiation stage, ranging from 1.0 to 27.2 per cent in 2022 and 1.0 to 21.0 per cent in 2023, with overall mean incidences of 11.30 and 9.77 per cent, respectively. Molecular characterisation confirmed the identity of the pathogen as *P. oryzae,* showing 99.59 per cent similarity with a reference isolate (ON506191) and deposited under accession number PX116867. Pathogenicity was proven on susceptible cultivar 'Himalayan 2216', where typical blast lesions developed 5-7 days after inoculation. The study provides updated spatial epidemiological information on rice blast in Himachal Pradesh and identifies stable hotspot districts and locations.

Keywords: Rice blast, *Pyricularia oryzae,* Per cent disease incidence

Rice (*Oryza sativa* L.) is the second most widely cultivated crop worldwide and an important cereal belonging to the family Poaceae. At present, rice ranks as the world's third most important cereal crop after maize and wheat (USDA 2025)[1] and serves as the staple food for more than half of the global population. Rice is nutritionally rich, providing about 80 per cent carbohydrates, 7-8 per cent protein, 3 per cent fat and nearly 3 per cent dietary fibre[2]. In India, rice is cultivated on approximately 51.4 million hectares, with an annual production of about 150.18 million tonnes[3]. In Himachal Pradesh, rice is cultivated over an area of about 66.17 thousand hectares with an annual production of 151.39 thousand tonnes and an average productivity of 2,288 kg/ha[4].

Rice production is severely constrained by both abiotic and biotic stresses. Among biotic factors, diseases caused by fungi, bacteria and viruses pose serious threats to rice productivity and quality. Major rice diseases worldwide include rice blast, bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*), sheath blight (*Rhizoctonia solani*), brown spot (*Bipolaris oryzae*), false smut (*Ustilaginoidea virens*) and rice tungro disease. Among all the rice diseases, rice blast caused by the fungus *Magnaporthe oryzae* is considered the most damaging disease in rice due to its high pathogen plasticity and mutation rate[5]. The disease alone accounts for 10-30 per cent yield losses globally, equivalent to food losses sufficient to feed nearly 60 million people annually[6,7].

*Author for correspondence

Manuscript Received: 08.01.2026; Accepted: 05.02.2026

The pathogen *Pyricularia oryzae* Cavara (teleomorph *Magnaporthe oryzae*) is a hemibiotrophic fungus that initially establishes a biotrophic relationship with the host and later switches to a necrotrophic phase, resulting in host tissue necrosis[8]. The pathogen completes its disease cycle within about a week, with new lesions appearing within 4-5 days under favourable environmental conditions, thereby facilitating rapid disease spread. Rice plants are susceptible to blast at all growth stages, with leaf blast predominating during the vegetative stage and neck blast during the reproductive stage. Neck blast is considered to be the most destructive phase, often causing severe yield losses even when leaf blast incidence is low[9].

In India, rice blast is the most frequent and destructive disease of irrigated rice in subtropical and temperate regions and poses a serious threat to national food security[10,11]. In Himachal Pradesh, rice blast occurs regularly in moderate to severe forms, particularly in the mid-hill agro-climatic zones comprising districts such as Kangra, Mandi and Chamba. Yield losses due to rice blast in the state have been reported to range from 5 to 25 per cent under natural field conditions, while severe epidemics may result in losses exceeding 40 per cent, particularly when neck blast infection coincides with the grain-filling stage[12,13]. Such losses significantly affect the income of small and marginal farmers who dominate rice cultivation in the hilly regions. Therefore, keeping in view the importance of crop and rice blast disease, the present study was undertaken to investigate the occurrence and pathogen characteristics of rice blast disease caused by *Pyricularia oryzae* under the mid-hill agro-climatic conditions of Himachal Pradesh.

MATERIALS AND METHODS

Collection of Diseased Leaf Samples and Isolation of Pathogen

A survey was carried out during the kharif season of 2022 and 2023 to study the incidence of rice blast disease in different rice-growing areas of Himachal Pradesh, India. The survey covered nine districts, *viz.,* Kangra, Mandi, Hamirpur, Chamba, Sirmaur, Bilaspur, Una, Kullu and Shimla, representing diverse agro-climatic conditions of the state. Representative locations were selected in each district. Both improved/high-yielding varieties (GBW 343, Vijeta series, Star 795, Sawa 200, Tanatan, LG No. 1, Chaina (LLR), etc.) and local landraces commonly grown by farmers were included in the study. Geographical details such as latitude, longitude and altitude of each location were recorded using GPS data and secondary sources to relate disease incidence with topographical variations. Rice blast caused by *Pyricularia oryzae* Cav. was identified in the field based on typical symptoms such as spindle-shaped to elliptical lesions with greyish centres and brown margins on leaves.

A roving survey method was adopted for recording disease incidence. Surveys were conducted at the maximum tillering to panicle initiation stage, when blast symptoms were clearly visible. In each selected field, plants were observed by following a zig-zag sampling pattern to ensure uniform coverage. The disease incidence was calculated using the formula given by McKinney[14]:

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected seedlings}}{\text{Total number of seedlings}} \times 100$$

Separate observations were recorded for the years 2022 and 2023 at each location. Year-wise disease incidence data were compiled location-wise and district-wise. The mean disease incidence for 2022 and 2023 was calculated for each location to assess overall disease intensity. District-wise averages were computed by pooling data across locations within each district. An overall mean disease incidence was calculated by pooling data from all surveyed districts to understand the general prevalence of rice blast in the state. Rice leaves showing typical blast symptoms were collected from infected fields and brought to the laboratory for isolation of the pathogen.

Isolation and Maintenance of the Pathogen

Small pieces (approximately 3-5 mm) were cut from the advancing margins of lesions, surface sterilised with 0.1 per cent Sodium hypochlorite for 30 seconds and rinsed thoroughly three times with sterile distilled

water. The sterilised leaf bits were blot-dried on sterile filter paper and placed on Oatmeal Agar (OMA) medium in Petri plates. The plates were incubated at 25±1°C for 14 days until fungal growth appeared. After sufficient sporulation, sterile distilled water was added to the culture surface and gently rubbed with a sterile glass rod to dislodge conidia. A dilute spore suspension was prepared so that individual conidia were well separated. For single spore isolation, a drop of the diluted suspension was spread evenly on the surface of water agar (2%) plates. The plates were incubated at 25±1°C for 12-18 hrs. to allow spore germination. Under a compound microscope, a single germinated conidium was identified and carefully picked using a sterile fine needle.

The selected single germinated spore was aseptically transferred to a fresh OMA plate and incubated at 25±1°C. After 5-7 days, uniform mycelial growth was observed, confirming the successful establishment of a single spore culture. The culture was sub-cultured periodically and maintained on PDA slants at 4°C for further studies.

Identification of the Pathogen

Morphological characteristics of the pathogen, including conidial shape, size and colony characteristics on OMA media, were recorded and compared with standard descriptions available in literature[15]. Different colony characters, *viz.,* colony colour and production of melanin was recorded. Also, conidial character, *viz.,* conidia shape, colour, septa and presence or absence of hilum was recorded.

Pathogenicity of the isolated fungus was proved by fulfilling Koch's postulates through pot culture under controlled conditions. Seeds of a susceptible rice cultivar (Himalayan 2216) were sown in plastic pots (10 cm diameter) filled with sterilised soil and farmyard manure (FYM) in a 2:1 ratio. The pots were kept in a net house and irrigated regularly. Plants were maintained under optimum growth conditions until the 3-4 leaf stage, which is highly susceptible to blast infection. The isolated pathogen, obtained through a single spore, was grown on oatmeal agar (OMA) at 25±1°C to induce profuse sporulation. After 10-12 days, sterile distilled water containing 0.02 per cent Tween-20 was added to the culture surface, and conidia were gently scraped using a sterile glass rod. The spore suspension was filtered through double-layered muslin cloth and adjusted to a concentration of 1×10^5 conidia/ml using a haemocytometer. The conidial suspension was sprayed uniformly on rice plants using a hand sprayer until runoff during the evening hours. Control plants were sprayed with sterile distilled water containing Tween-20 only. Immediately after inoculation, pots were covered with transparent polythene bags or kept in a humid chamber to maintain high relative humidity (>90%) for 24-48 hours, which is essential for infection.

After the initial incubation period, polythene covers were removed, and plants were maintained at 25±2°C, with relative humidity of 80-90 per cent. Light irrigation was provided to avoid washing off the inoculum. Plants were regularly observed for symptom development. The pathogen was reisolated from freshly developed lesions following the same isolation procedure as described earlier. The reisolated fungus was compared morphologically with the original culture and found to be identical, thus confirming the pathogenic nature of the isolate.

RESULTS AND DISCUSSION

Disease Survey and Collection of Rice Blast Samples

A comprehensive field survey was undertaken during the kharif seasons of 2022 and 2023 to assess the incidence of rice blast disease across major rice-growing districts of Himachal Pradesh (Figure 1). Disease incidence was recorded from multiple locations within each district, representing a wide range of altitudes and agro-climatic conditions.

During 2022, rice blast incidence varied considerably among districts, reflecting differences in environmental suitability for disease development (Table 1). The highest mean disease incidence was recorded in Kangra district (16.16%), followed by Mandi (15.80%) and Chamba (14.00%). These districts

generally experience warm and humid conditions during the crop-growing season, which are highly favourable for blast development. Moderate disease incidence was observed in Kullu (12.67%) and Sirmaur (8.00%), whereas relatively lower incidence was recorded in Shimla (7.40%), Hamirpur (5.14%), Bilaspur (5.00%) and Una (4.00%). The overall mean disease incidence across the state during 2022 was 11.30 per cent. At the location level, the highest disease incidence in 2022 was recorded at Bhagotla in Kangra district (27.22%), indicating severe disease pressure under highly favourable microclimatic conditions. In contrast, the lowest incidence was observed at Mehatpur in Una district (1.00%), followed by several locations in Hamirpur and Bilaspur, where disease incidence remained below 5 per cent.

In 2023, a general decline in rice blast incidence was observed across most districts, although the disease remained widespread. Kangra (13.56%), Mandi (12.90%) and Chamba (11.13%) continued to experience comparatively higher disease incidence, suggesting persistence of favourable microclimatic conditions and possible carryover of inoculum. Lower mean disease incidence was recorded in Sirmaur (10.75%), Kullu (9.50%), Shimla (5.80%), Hamirpur (5.07%), Bilaspur (3.75%) and Una (3.22%). The overall mean disease incidence during 2023 was 9.77 per cent. Location-wise analysis revealed that the highest disease incidence during 2023 was recorded at Bhagotla (Kangra, 21.00%) and Dhaula Kuan (Sirmaur, 18.00%), followed by Balh in Mandi district (16.50%). Conversely, the lowest incidence was observed at Dhundla in Una district (1.00%), with several locations in Hamirpur and Bilaspur registering disease incidence in the range of 2–3 per cent.

The clear gradient from higher blast incidence in mid-hill districts (Kangra, Mandi, Chamba, Kullu, Sirmaur) to lower incidence in low-hill/foothill (Una, Bilaspur) and high-hill (parts of Shimla) zones corroborates the established influence of altitude-linked temperature and humidity regimes on blast epidemiology. Studies from mid-hills of Himachal Pradesh and other Himalayan regions have shown that altitudes around 800-1500 m with warm days, cool-humid nights and frequent dew formation create ideal conditions for conidial germination and sporulation, whereas hotter low hills or cooler high hills often fall outside the optimum thermal window for explosive epidemics[16,17,18].

The widespread yet spatially heterogeneous distribution of blast observed in this study reinforces the need for location-specific disease forecasting and targeted management strategies in Himachal Pradesh. Earlier work from Palampur and Kangra has demonstrated the feasibility of deriving simple weather-based blast prediction rules and using them to guide timely fungicide applications and deployment of resistant cultivars, thereby reducing disease severity under mid-hill conditions[16,18,19].

Recent spatial modelling from Karnataka and climate-impact assessments from Nepal further highlight that climate change-driven shifts in rainfall and humidity patterns may expand or intensify blast hotspots, making continued surveillance and dynamic risk mapping essential for hill ecosystems[11,17,18].

Morphological Identification

The isolate obtained from blast lesions produced initially white to grey, cottony colonies on oatmeal agar that turned dark grey to olivaceous to black with age, with a dark reverse side due to melanin, which is typical of *P. oryzae*. Conidia were hyaline to pale olivaceous, smooth, predominantly pyriform and 3 celled (2 septa) with a narrow apex, broad base and distinct hilum (Table 2). The colony characters observed on artificial medium showed close conformity with those described for *Pyricularia oryzae* in standard literature[15]. The colonies were initially white to greyish in colour and gradually turned dark grey to olivaceous or black with age. Such age-dependent colour change is a characteristic feature of *P. oryzae* and is mainly attributed to the production and accumulation of melanin in the mycelium, which also imparts a dark appearance to the reverse side of the culture. Similar colony morphology, including cottony to fluffy growth with dark pigmentation on the reverse, has been reported by Veeraraghavan and Padmanabhan[20] and

Table 1. Status of rice blast in Himachal Pradesh

District	Location	Variety	Latitude (°N)	Longitude (°E)	Altitude above Sea Level (m)	Disease Incidence (%)		Average Disease Incidence (%)
						2022	2023	
Kangra	Panchrukhi	GBW343, Local	32.0957	76.5528	1,300	21.50	17.00	19.25
	Gopalpur	6129	32.0950	76.5300	1,260	18.00	16.50	17.25
	Bhagotla	Local	32.0330	76.4650	1,000	27.22	21.00	24.11
	Latwala	GBW343	32.0900	76.5500	1,250	15.00	15.00	15.00
	Baijnath	Tanatan	32.0521	76.6493	998	21.50	15.00	18.25
	Mahakaal	Tanatan	32.0500	76.6500	1,020	15.00	13.60	14.30
	Sihotu	HS562, 5258	32.0980	76.5250	1,230	12.50	12.44	12.47
	Pudwa	HS562, 5258	32.0850	76.5250	1,240	15.40	12.00	13.70
	Parour / Paror Khas	Local	32.0837	76.4549	970	18.00	14.70	16.35
	Mehnja	Sawa 200	32.1050	76.5100	1,200	18.00	16.50	17.25
	Narwana (near Yol)	Sawa 200	32.1000	76.2200	1,050	13.00	12.50	12.75
	Tapovan	Tanatan	32.1100	76.5300	1,240	15.00	11.00	13.00
	Gaggal	Sawa 200	32.1644	76.2624	759	12.00	10.00	11.00
	61 Mile	Star 795	32.2100	76.3400	850	9.50	6.50	8.00
	Sheela Chowk	Star 795, Sawa 200	32.1967	76.3436	900	18.00	16.00	17.00
	Average					16.16	13.56	14.86
Mandi	Baldwara	LV	31.7675	76.7186	810	12.00	12.00	12.00
	Bhanbla Bahi	Tanatan	31.5952	76.7412	775	6.50	6.00	6.25
	Narla	Sawa 200	31.5600	76.7400	800	18.00	12.00	15.00
	Harabagh	Tanatan	31.9539	76.8846	900	18.00	18.00	18.00
	Chhang	Star 795	31.5600	76.7300	800	17.50	16.00	16.75

Table 1 Continued

	Barot	LV	32.0173	75.7656	1,829	12.00	10.00	11.00
	Ghatasani	Chaina (LLR)	31.9539	76.8846	900	18.00	12.50	15.25
	Chauntra	Chaina (LLR)	32.2200	76.7200	1,300	17.00	14.00	15.50
	Ropapadhar	Chaina (LLR)	31.6000	76.8500	900	18.00	12.00	15.00
	Balh	Byala	31.8000	76.8700	1,080	21.00	16.50	18.75
	Average					**15.80**	**12.90**	**14.35**
Hamirpur	Bharedi / Bhareri	Local	31.7070	76.6200	800	9.00	7.00	8.00
	Dimmi	LG No.1	31.64296	76.64271	780	3.00	3.00	3.00
	Khadd Bazaar	Star 795	31.6900	76.5300	780	6.00	9.00	7.50
	Mohin	Star 795	31.6855	76.5365	785	3.00	2.50	2.75
	Kehdru	LG no. 1	31.6500	76.6400	800	3.00	3.00	3.00
	Bhida / Bhadru	Sawa 200	31.6430	76.6450	780	3.00	5.00	4.00
	Jhinjkari	Local	31.6400	76.6500	780	9.00	6.00	7.50
	Average					**5.14**	**5.07**	**5.11**
Chamba	Chuari Khas	Local	32.4319	76.0120	1,000	16.50	12.50	14.50
	Sahala	Vijeta 100	32.3035	76.1961	940	16.00	12.00	14.00
	Sihunta	Vijeta 700	32.2931	76.0609	942	6.00	6.00	6.00
	Thulal	Vijeta 100	32.2931	76.0609	940	17.50	14.00	15.75
	Average					**14.00**	**11.13**	**12.56**
Sirmaur	Paonta Sahib	Local	30.4367	77.6100	389	6.00	4.00	5.00
	Kiyarda	Local	30.4800	77.5700	400	8.00	6.00	7.00
	Pipliwala	Local	30.4800	77.5700	400	6.00	6.00	6.00
	Dhaula Kuan	Local	30.7988	77.1217	470	12.00	18.00	15.00

Table 1 Continued

	Kolar	Local	30.5022	77.4197	520	12.00	16.50	14.25
	Tokion	Local	30.5000	77.4200	500	6.00	6.00	6.00
	Surajpur	Local	30.5000	77.4200	520	6.00	12.00	9.00
	Average					**8.00**	**10.75**	**9.38**
Bilaspur	Deoli	Vijeta 100	31.3377	76.7864	375	5.00	3.00	4.00
	Namhol	Local	31.2569	76.8598	1,208	6.00	6.00	6.00
	Geharwin	Local	31.3905	76.6772	820	3.00	3.00	3.00
	Average					**5.00**	**3.75**	**4.38**
Una	Gagret	Vijeta 100	31.6700	76.0700	439	3.00	3.00	3.00
	Dhundla	Star 795	31.5492	76.4156	400	3.00	1.00	2.00
	Haroli	LG no. 1	31.4232	76.2319	407	4.00	3.00	3.50
	Mehatpur	Sawa 200	31.3981	76.3450	389	1.00	3.00	2.00
	Average					4.00	3.22	3.61
Kullu	Naggar	Local	32.1139	77.1643	1,712	18.00	9.00	13.50
	Hallan / Hallan-I	Local	32.1494	77.2520	1,189	11.00	7.50	9.25
	Lug Valley (central)	Local	32.1500	77.2500	1,800	9.00	12.00	10.50
	Average					**12.67**	**9.50**	**11.08**
Shimla	Peja	Local	31.1740	77.9400	1,450	11.00	9.00	10.00
	Jangal (Ganoti)	Local	31.1730	77.8880	1,520	9.00	6.00	7.50
	Daboli / Bhadoli	Local	31.1714	77.6801	1,550	6.00	5.00	5.50
	Kaloti	Local	31.2306	77.80595	1,733	8.00	6.00	7.00
	Ganvi	Local	31.4715	77.6843	1,742	3.00	3.00	3.00
	Average					**7.40**	**5.80**	**6.60**
Grand Mean						11.30	9.77	10.54

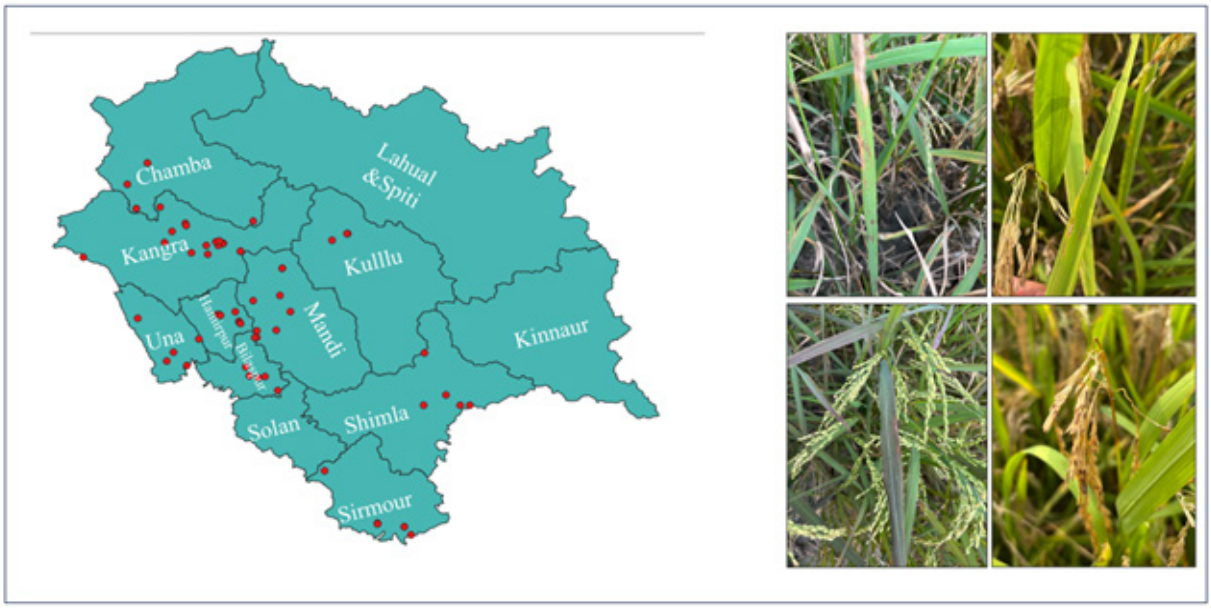


Figure 1. Distribution of rice blast in major rice-growing areas of Himachal Pradesh.

Ou[21], who described greyish to olivaceous colonies of *P. oryzae* on potato dextrose agar and other media.

Microscopic examination of the conidia revealed that they were characteristically pyriform (pear-shaped), hyaline to pale olivaceous in colour and predominantly 2-septate, forming three distinct cells (Figure 2). The basal cell was broader and possessed a short hilum. These features are considered diagnostic for P. oryzae and are in close agreement with earlier descriptions. Ou[21] reported that the conidia of P. oryzae are typically pyriform, smooth, hyaline and mostly three-celled, although one- or two-septate conidia may occur depending on growth conditions. Ellis[22] also described the conidia as pear-shaped, slightly curved, hyaline and smooth, with one to two septa, thereby supporting the present observations.

A comparative analysis of the isolated pathogen with descriptions available in the literature further strengthened the identification. The shape of the conidia in the present isolate was pyriform and elongated, which closely matches the pear-shaped and slightly curved conidia described in literature (Cavara 1891). The predominance of 2-septate conidia observed in the present study falls within the range of one to two septa commonly reported for *P. oryzae*[21,22]. The conidial apex was narrow and acute, while the base was broad, which is in agreement with the literature descriptions of an acute or pointed apex and a rounded basal cell[20].

The conidia observed in the present investigation were hyaline with a smooth surface, which is a characteristic feature of *P. oryzae* and has been consistently reported by several workers[22,23]. Furthermore, the conidia were borne singly with a distinct hilum at the basal end. Literature indicates that *P. oryzae* produces conidia singly on simple conidiophores, and the conidia possess a truncate or short-toothed basal hilum, which serves as the point of attachment[21,23]. The presence of a short hilum in the present isolate is therefore in complete agreement with standard taxonomic descriptions.

Overall, the close agreement between the colony characters and conidial morphology of the isolated pathogen with those described in earlier literature confirms the identity of the fungus as *Pyricularia oryzae* (Cavara)[24]. These morphological observations provide a reliable basis for pathogen identification and form a sound foundation for subsequent pathogenicity tests and further studies on the epidemiology and management of rice blast.

Table 2. Morphological identification of *Pyricularia oryzae* (Cavara)[15]

Characteristic	Isolated Pathogen (Spore)	Description in Literature
Shape	Pyriform (elongated)	Pyriform (pear-shaped), slightly curved
Septation	2 septa (3 cell)	1 - 2 septum (mostly)
Apex	Narrow	Acute or pointed
Base	Broad	Rounded
Color	Hyaline	Hyaline (transparent)
Surface	Smooth	Smooth
Attachment	Singly borne, hilum present	Produced on simple conidiophores singly or in small clusters, with a truncate or shorttoothed base (hilum)

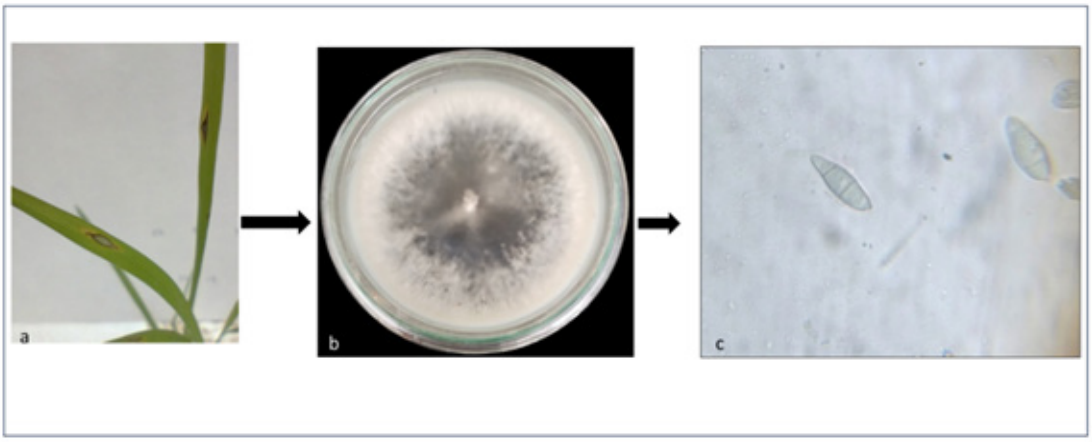


Figure 2. **(a)** Boat-shaped lesions on leaves of rice; **(b)** Isolation of pathogen on oatmeal agar medium; **(c)** Conidia of *Pyricularia oryzae* under microscope.

Pathogenicity Test

Typical blast symptoms, characterised by spindle-shaped to elliptical lesions with greyish centres and brown margins, appeared on leaves 5-7 days after inoculation. No symptoms were observed on control plants. The pathogen was reisolated from freshly developed lesions. The reisolated fungus was compared morphologically with the original culture and found to be identical, thus confirming the pathogenic nature of the isolate. The development of typical blast symptoms on inoculated plants, absence of symptoms in control plants and successful re-isolation of *P. oryzae* fulfilled Koch's postulates, thereby confirming the pathogenicity of *Pyricularia oryzae* on rice under pot culture conditions. Similar symptom expression and incubation period have been widely reported in earlier studies, thereby supporting the present findings[21,23]. Earlier workers have emphasised that symptom development exclusively on inoculated plants, along with healthy control plants, is a critical requirement for the confirmation of pathogenicity of *P. oryzae*[25,26]. Similar successful re-isolation and confirmation of *P. oryzae* from artificially inoculated plants have been

reported by Ou[21], Wilson *et al.*,[27] and Wang *et al.*,[28] who observed that the re-isolated cultures closely resembled the original pathogen in both cultural and morphological traits.The incubation period of 5-7 days observed in the present study is also in conformity with earlier reports, which indicate that *P. oryzae* typically produces visible leaf blast lesions within 4-7 days under favourable temperature and humidity conditions[21,23]. Such rapid symptom development reflects the aggressive nature of the pathogen and its high infective potential under conducive conditions.

CONCLUSIONS

These findings underscore that rice blast remains a major biotic constraint to rice production in Himachal Pradesh, threatening food security and farmer livelihoods. The persistent high incidence in mid-hill districts necessitates location-specific disease forecasting systems and targeted management strategies, including timely fungicide applications and deployment of resistant cultivars adapted to local conditions. Continued surveillance and dynamic risk mapping are essential, particularly considering climate change-driven shifts that may intensify blast hotspots in these vulnerable hill ecosystems. Future research should focus on developing integrated disease management protocols and identifying durable resistance sources for sustainable rice production in the region.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest among the authors in this research paper.

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AUTHORS CONTRIBUTION

AP: conducted survey, analysed data and wrote the original manuscript. PK: conceptualize the research programme, methodology, provided resources, reviewed and edited the manuscript. T and A: reviewed and edited the final draft.